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Research Article

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Assessment of stress biomarkers responses in mantle and adductor muscles of *Mactra stultorum* following lead exposure

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Abstract

The objective of the present work is to evaluate the possible toxic effect engendered by graded doses of lead chloride ($PbCl_2$) on *Mactra stultorum* mantle and adductor muscles through a battery of biomarkers responses. *M. stultorum* were divided into 4 groups and exposed to three concentrations of $PbCl_2$ (D1:1mg/L, D2: 2.5 mg/L and D3: 5 mg/L) with control during five days. Our findings showed decreases of lipid contents in both organs following $PbCl_2$ exposure, while, proteins declined only in the adductor muscles of the treated *M. stultorum.* During our experiment, the $PbCl_2$ exposure induced the levels of metallothionein (MTs), malondialdehyde (MDA) and advanced oxidation protein products (AOPP) in both organs as compared to the control. These biomarkers responses are distinctly different between mantle and adductor muscles.

Keywords: Lead chloride, *Mactra stultorum*, Mantle, Adductor muscles, Biomarkers responses.

Introduction

The contamination of aquatic ecosystems by several environmental pollutants has become a worldwide problem in the last years [1]. The presence of heavy metals in those environments and their accumulation in marine organisms has been largely investigated during the last decades because of their harmful effects and persistence [2]. For the global environmental health, lead (Pb) is considered to be a major hazard. This non-essential and toxic heavy metal is the most abundant metal in the aquatic system. In nature, it is present as a divalent cation and principally forming stable complexes with sulfur. It has a natural origin or it is realized from many industrials discharges such as lead ore mining and smelting, refining, alkyl-lead petroleum combustion, batteries and cement manufacture [3]. At the national level, lead is one of metals contaminating the Tunisian coasts because of its highest concentrations and has been considered a major source of pollution in Tunisian waters [4,5]. Thus, high Pb levels which are exceeding the permissible limit (1 mg/kg), [6] have been recorded in Tunisian bivalves tissues (values comprised between 5 and 9 mg/kg DW) [5,7]. Moreover, their accumulation in aquatic ecosystems can become dangerous to all kinds of organisms including bivalves, fishes, aquatic plants and human life, causing many toxic effects [8].

Mactra stultorum is considered as ecologically important components of marine environments and an important edible marine bivalve due to their richness of protein and essentials fatty acids [9, 10]. It has a wide distribution along the Mediterranean and Atlantic coasts and estuaries [11]. So, it is abundant specie in the subtidal area and shallow seas along the coast of Tunisia [12]. Moreover, due to their benthic and sedentary mode of life; suspension-feeding mode and high filtration rate, it is easily exposed to environmental pollution [13]. *M. stultorum* can accrued a enormous number and high concentration of heavy metals in their tissues [14,15,16]. Therefore, similar to other species of *Mactra*, Mussels and Oysters, *M stultorum* can be used as a good bioindicator of heavy metal pollution in marine environments [17].

The excessive accumulation of lead in the bivalve tissues can induce oxidative stress through the overproduction of reactive oxygen species (ROS) in the cells which affect cellular functions [18,19]. So, when these increases of ROS levels get over the scavenging capacity of organisms, the superfluous free radicals may advantage to oxidative damage in basic biological molecules, such as lipid peroxidation, protein oxidation and DNA damage [18,19,20].

Thus, the lipid peroxidation constitutes an involvement of self-propagating sequence of chemical reactions that occurred in the bulk phase of cell membrane lipid bilayers. Malondialdehyde (MDA) and 4-hydroxyalkenals are the degradation products of lipid peroxidation and their levels reflect the degree of oxidative damage and constitute a biomarker specific to environmental stresses [21].

Moreover, lipid peroxidation and other damages resulting from metal toxicity are modulated by antioxidant systems and stress proteins such as metallothioneins (MT) [22]. These systems of defense play a key role in the alive organisms which can provide for the different cells the protection against environmental toxicity control metabolism homeostasis [22]. Metallothionein (MT) is a low-molecular-weight and cysteine-rich protein, identified for the first time in the kidney of the horse [23]. MT's plays a crucial role in metal metabolism and principally in the detoxification mechanisms as a metal-chelating agent for the excess of metals in the cells [24]. Further, it ensure an essential role in immune response [25], antioxidant processes [26,27], and response to estrogenic compounds [28]. In aquatic environments, MT has been implied to be used as a bioindicator for metal contamination because of its possibility to bind to particular metals.

Even though there are investigations about the impact of $PbCl_2$ on *M. stultorum* gills and digestive gland [15,16], still information are lacking about the impact of this metal on the metabolism and redox status of other organs. Thus, in this study, we focused on identifying the metabolic and redox strategies developed by *M. stultorum* adductor muscles and mantle to cope with graded $PbCl_2$ concentrations.

Materials and Methods

Experimental protocol

Mature clams individuals (Shell length (SL): 3.5 ± 0.63 cm and Total weight (TW) 8.03±0.47 g) were collected at 1 m depth by scuba divers from the Bizerte lagoon. After sampling, clams were acclimated for 7 days in aquaria (20 L). The water was daily renewed and physicochemical parameters were controlled (temperature (18°C), salinity (30 psu), pH (7.4 ± 0.2), and photoperiod (12h/12h)). During the acclimation period, green microalgae *Isochrysis affinis galbana* (t-ISO, 2 million cells per ml) was fed regularly to *M. stultorum*. At the end of the acclimation period, clams were divided in 4 groups of 18 clams. Each group was placed in 8 L plastic aquaria and was represented in triplicate (n=6 clams per replicate). Clams first group was kept in aquaria$_1$ containing filtered natural seawater (control), while other groups were exposed for 5 days to different concentrations of unmixed $PbCl_2$ metal (Lead chloride; $PbCl_2$; Sigma-Aldrich; powder 98%) which was dissolved in pure water. During metal exposure, clams were exposed to graded concentrations of $PbCl_2$ as follows: aquaria$_1$: control; aquaria$_2$: 1mg/L; aquaria$_3$: 2.5mg/L and aquaria$_4$: 5mg/L with controlled conditions as mentioned above (**Figure 1**) and no added food. Half (50%) aquaria water volume was replaced every 24 h in order to maintain the water quality, and concentrations of $PbCl_2$ were reestablished. $PbCl_2$ concentrations were selected based on previous trials achieved on other bivalves [29,30]. During the experimental period, no mortality has been reported.

Preparation of the samples for biochemical analyses

After $PbCl_2$ exposure, clams were sacrificed and the mantle and adductor muscle were quickly removed and rinsed with cold distilled water in order to remove the externally bound Pb. 6 replicate of each group tissues (mantle and adductor muscle; n=3 for each replicate) were homogenized in a Tris-HCl buffer (20mM; pH=7.4) in cold conditions, then centrifuged at 10.000 × g for 20 min (4°C). Tissues supernatants were stored at -80°C for oxidative stress parameters analysis.

Biochemical analyses

For biochemical analysis, chemicals were purchased from local commercial suppliers. Except for 5,5-dithio-bis-(2- nitrobenzoic acid) (DTNB) and 2-thiobarbituric acid) (TBA) they were purchased from Sigma chemical Co (Saint Louis, MO 63103, USA).

Protein quantification

Based on Lowry *et al.* [31] method, protein content was estimated using Folin Reagent and bovine serum albumin (BSA) as a standard. 2 ml of a solution mixture (sodium carbonate hydrate dissolved in a solution of NaOH (0.1N), copper sulfate and sodium hydrate dissolved in water) were added to 10 µl supernatants. Then, 200 µl of Folin Reagen was added to the formed mixture for reaction activation. After 30 min incubation, protein content was determined at 540nm using the spectrophotometric method.

Lipid quantification

According to the method of Goldsworthy *et al.* [32], lipids were determined and the extraction was carried out following Shibko *et al.* [33] method. 0.5 g of tissues are removed, cut and macerated in 10 ml of trichloroacetic acid (TCA, 20%). After grinding and filtration and a first centrifugation 5000× g for 10 min, the pellet is kept in the same tube which we added 1 ml of the mixture Ether / Chloroform (1 v/ 1v). Subsequently, the last volume is submitted to second centrifugation 5000× g for 10 min and 100 µl of the supernatant is taken which we added 1 ml of sulfuric acid. And after stirring, the tubes were placed in a hot water bath (100 °C) for 10 min. Then, we added 2.5 ml of the sulfophospho-vanillin mixture (85%) to 200 µl of the extract

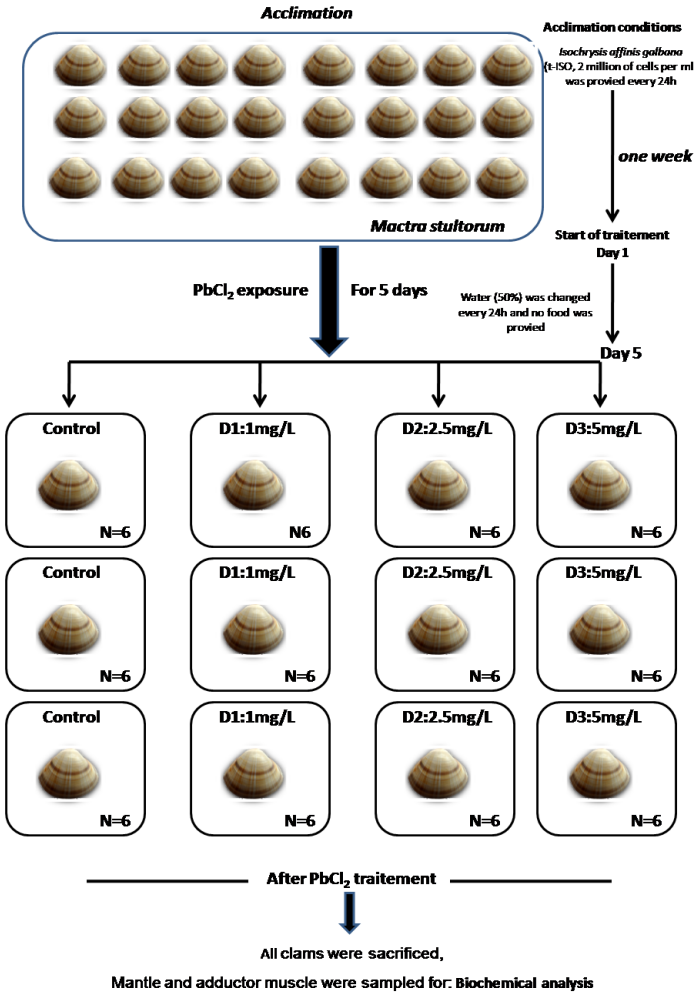

Figure 1. Experimental conception of *Mactra stultorum* exposed to graded doses of lead chloride ($PbCl_2$).

which was incubated for 30 minutes in a darkroom. Lipid quantities are determined by the spectrophotometric method at 530 nm. A calibration range was carried out from a stock solution prepared from sunflower oil. The lipid contents are expressed in mg/g wet weight (mg/gWW)

Malondialdehyde (MDA) measurement

MDA level was determined according to Draper and Hadley [34]. An aliquot of 0.5ml of each tissue supernatant was incubated for 1 hour in heated water (37°C) and mixed with 0.5 ml of trichloroacetic acid (TCA 30%). After centrifugation for 10 min (3500× g/4 °C), we added 500µl of thiobarbituric acid (TBA 0.67%) to 0.5 ml of supernatant. After incubation for 10min, MDA levels were determined by spectrophotometric method at 532 nm and expressed as nmol /mg protein.

Advanced oxidation protein products level (AOPP) measurement

The advanced oxidation protein products (AOPP) levels were determined following the method of Kayali *et al.* [35]. After protein precipitation in double volumes of phosphate buffer (0.1M; pH = 7.4). Then, potassium iodide (1.16M) and absolute acetic acid (200µl) were added to clams supernatants. We used the extinction coefficient of 261 cm^{-1} mM^{-1} for AOPP quantification. AOPP levels were determined at 340 nm and expressed as µmol/mg of protein.

Metallothionein (MTs) content

According to the method of Viarengo *et al.* [36] modified by Petrovic et al. (2001), MTs were determined. The supernatant of each tissue from each group (500µl) was mixed with ethanol/chloroform solution (95%; 1%). After centrifugation for 6000 x g during 10 min in cold, EDTA (1mM) and NaCl (0.25M) were added to the pellets and MTs absorbance was measured at 412nm. MTs were expressed as nmol GSH/mg protein.

Statistical analysis

Statistica software version 5.0 was used for statistical analysis. The homogeneity and normality of variables were tested using the Shapiro-Wilcoxon test. Differences between variables

were assessed by One-way ANOVA and deemed significant at $p < 0.05$. For each parameter, results were expressed as means ± standard deviation (SD). Pearson correlation matrix and principal component analysis (PCA) were used to discriminate significant correlations between biochemical parameters.

Results

The $PbCl_2$ effects on general behavior of *M. stultorum*

No mortality was noticed in treated clams by $PbCl_2$ different doses. During the experimental period, clams behavior was regularly monitored (e.g. filtration activity, reduced respiration, siphon retraction…). Those parameters remained stable even in clams exposed to the high dose of $PbCl_2$ (D3).

Estimation of total protein contents

Results showed no significant variation in protein content of clam's mantle tissues after $PbCl_2$ treatment. However, significant decreases in the amount of total protein were revealed in the adductor muscles of *M. stultorum* following lead exposure (-23%, -24% and -20% in clams exposed to doses D1; D2 and D3 respectively compared to controls (**Table 1**).

Estimation of lipid contents

Lipids contents decreased significantly in clams mantle and adductor muscle tissues after 120 hours of exposition to $PbCl_2$. Compared to the control, this decline was recorded for the mantle by -37%, -60% and -67% in clams exposed to 1mg/L; 2.5mg/L and 5mg/L respectively) and for the adductor muscles by -32 % and -60% in clams treated by D2 and D3 respectively (**Table 1**).

Estimation of malondialdehyde (MDA) levels

Results showed that the progressive accumulation of Pb in both *M. stultorum* tissues induced lipid peroxidation in all treated clams by $PbCl_2$ which was revealed by MDA levels enhancement. In the mantle tissues and compared to the control, MDA levels increased with graded doses (D1, D2 and D3, respectively) by 57%, 150% and 575%. We also noted that MDA levels in the adductor muscles increased significantly by 58% and 74%, in clams treated with the highest doses (D1 and D2) (**Figure 2**).

Estimation of advanced protein oxidation products (AOPP) levels

Significant increases in AOPP levels were recorded in the mantle of all $PbCl_2$ treated groups (1, 2.5 and 5 mg/L $PbCl_2$) with a dose dependent manner (+60, +65and +205% respectively). While in the adductor muscles tissue and compared to control, the increase of AOPP levels was observed only for clams exposed to 2.5mg /L; (+102%) and 5mg / L; (+132%) (**Figure 3**).

Estimation of metallothionein (MT's) levels

MT's levels increased significantly only in clams mantle tissues exposed to the highest dose of $PbCl_2$ (5mg / L; 163%) during 5 days. Furthermore, the MT's levels increased significantly in the adductor muscles with a dose dependent manner (+30, +45 and +268%) with D1, D2 and D3 $PbCl_2$ doses compared to control (**Figure 4**).

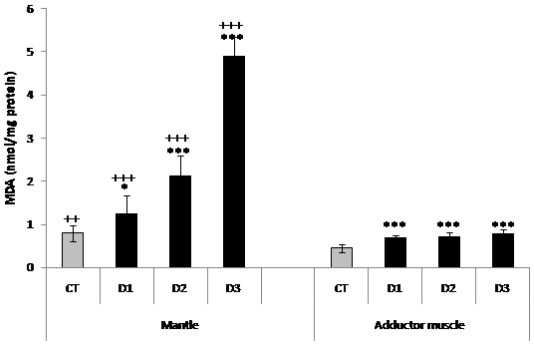

Figure 2. The MDA levels in the control and treated *M. stultorum* mantle and adductor muscles with Pb Cl_2 graded doses (D1, D2, D3) during 5 days. Values are expressed as means ± SD, 6 replicate in each group and tissues (n=3 clams). Pb Cl_2 graded doses: D1 (1mg/L Pb Cl_2); D2 (2.5mg/L Pb Cl_2); D3 (5mg/L Pb Cl_2). *** P <0.001: Pb Cl_2 groups VS controls for each tissue. ++ <0.01; +++ P <0.001: mantle VS adductor muscles for each condition.

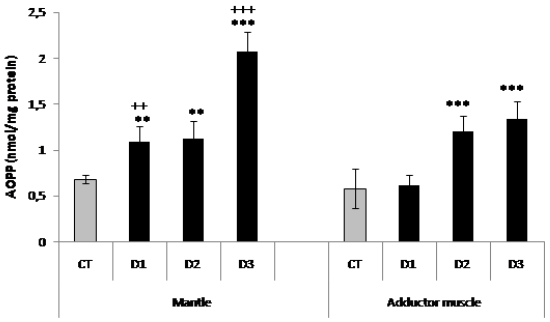

Figure 3. The AOPP levels in the control and treated *M. stultorum* mantle and adductor muscles with Pb Cl_2 graded doses (D1, D2, D3) during 5 days. Values are expressed as means ± SD, 6 replicate in each group and tissues (n=3 clams). Pb Cl_2 graded doses: D1 (1mg/L Pb Cl_2); D2 (2.5mg/L Pb Cl_2); D3 (5mg/L Pb Cl_2). ** P <0.01;*** P <0.001: Pb Cl_2 groups VS controls for each tissue. ++P <0.01; +++ P <0.001: mantle VS adductor muscles for each condition.

Principal component analysis (PCA) and correlation matrix

Correlation matrix and PCA were established in order to understand in the first time the effect of lead graded doses on stress biomarkers in mantle and adductor muscles tissues of *Mactra stultorum* and in second time to compare the response of these biomarkers between these tissues (**Figure 5** and **Table 2**). The first two factorial axes that explain 90.96% of the total variance (**Figure 5**). Factor 1 (56.38%) was characterized by high MDA and MT's levels (**Figure 5**). Factor 2 (33.37 %) was characterized by AOPP levels. Results showed that protein and lipid contents are intermediates compounds for F1 and F2. PCA results showed that there were two significant separations, the first one between controls of mantle and adductor muscles and the treated groups and the second one between both tissues from all treated groups (**Figure 5**). Control mantle and adductor muscles were projected in the positive sides of two factorials axes,

Table 1. Protein and Lipid contents in the control and treated *M. stultorum* mantle and adductor muscle with $PbCl_2$ graded Doses (D1. D2. D3) during 5 days. Values are expressed as means ± SD, 6 replicate in each group and each tissues (n=3 clams.). a: nmol/mg protein. b: mg/mg protein. Pb Cl_2 graded doses: D1 (1mg/L Pb Cl_2); D2 (2.5mg/L Pb Cl_2); D3 (5mg/L Pb Cl_2). ** P <0.01;*** P <0.001: Pb Cl_2 groups VS controls for each tissue. +++P <0.01; +++ P <0.001: mantle VS adductor muscles for each condition.

		CT	D1	D2	D3
Protein[a]	Mantle	$10.4\pm 0.67_{+++}$	$8.37\pm 0.76_{+++}$	$9.84\pm 0.64_{+++}$	$7.84\pm 0.69_{+++}$
	Adductor muscle	43.4 ± 4.91	$33.4\pm 2.02^{*}$	$32.8\pm 1.94^{*}$	$34.7\pm 5.14^{*}$
	Mantle	$4.2\pm 0.92_{++}$	$2.62\pm 0.71^{*}{}_{+++}$	$1.67\pm 50.27^{*}{}_{+++}$	$1.42\pm 0.32^{*}{}_{++}$
Lipid[b]	Adductor muscle	5.92 ± 0.35	5.89 ± 0.26	$4.02\pm 0.28^{*}$	$2.37\pm 0.83^{*}$

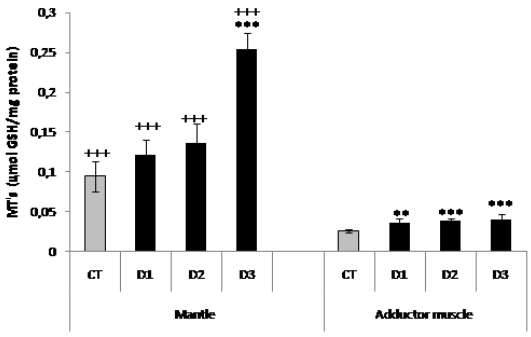

Figure 4. The MT's levels in the control and treated *M. stultorum* mantle and adductor muscles with Pb Cl_2 graded doses (D1, D2, D3) during 5 days. Values are expressed as means ± SD, 6 replicate in each group and tissues (n=3 clams). Pb Cl_2 graded doses: D1 (1mg/L Pb Cl_2); D2 (2.5mg/L Pb Cl_2); D3 (5mg/L Pb Cl_2). ** P <0.01;*** P <0.001: Pb Cl_2 groups VS controls for each tissue. +++ P <0.001: mantle VS adductor muscles for each condition.

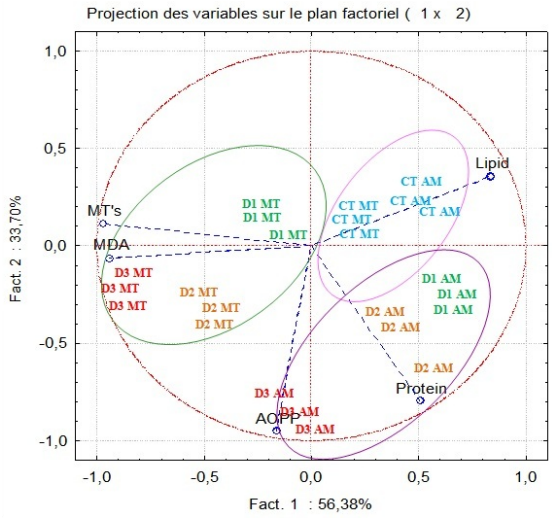

Figure 5. Principal analysis component (PCA) represented by two factors F1 and F2 and produced by biochemical variables in control and *stultorum* mantle and adductor muscles with Pb Cl_2 graded doses (D1, D2, D3) during 5 days. Projection of the variables and the cases on the factor-plane (1×2); D1:1mg/L $PbCl_2$; D2:2.5mg/L $PbCl_2$; D3:5mg/L $PbCl_2$; MT: mantle; AM: adductor muscles.

explaining by the high contents of lipids and protein and minor levels of lipid peroxidation and MT's. Second group was dominated by treated mantle clams by $PbCl_2$ which represented the negative side of F1 and the positive side of F2; revealing important lipid and protein oxidation. The third one including the adductor muscles from *M. stultorum* from all treated groups was characterized by a minor response of stress biomarkers comparing to mantle tissues and by a remarkable decrease in protein and lipid contents especially for clams exposed to high dose. Clearly, biomarkers responses involved in oxidative stress were significantly enhanced in both tissues clams treated with high $PbCl_2$ dose when compared to control groups.

Table 2. Correlation analysis (Pearson correlation) between the biochemical parameters in the control and treated *M. stultorum* mantle and adductor muscle with Pb Cl_2 graded doses (D11.D2. D33 during 5 days. Correlation coefficients statistically significant (p <0.05) . ns: not significant (p <0.05).

	Mantle				Adductor muscles			
Protein	Lipid	MDA	AOPP	Protein	Lipid	MDA	AOPP	
Lipid	ns				ns			
MDA	ns	-0.98			0.98	ns		
AOPP	ns	ns	0.98		-0.95	-0.96	ns	
MT	ns	ns	0.99	0.99	0.97	ns	0.99	ns

Pearson correlation matrix showed that protein contents in adductor muscles is significantly negatively correlated (p<0.05) with AOPP levels. While, lipid contents in mantle showed a negative correlation with MDA levels (p<0.05). Positive correlation was recorded between AOPP, MDA and MTs levels for mantle tissue. While, negative correlation was observed between AOPP levels and lipid contents for adductor muscles tissue. Thus, MT's presents in this tissue positive correlation only with MDA and protein levels (**Table 2**).

Discussion

Biological responses in native indicator species can give total and pertinent information on the potential impact of metal toxicity on ecosystem dynamics. Heavy metals constitute a group among environmental pollutants because of their bioaccumulation and non-degradable property. Lead is a non-essential metal, abundant in the oceans and exhibits widely regarded toxic effects associated with the stimulation of radical processes [37].

Lead is accumulated by different organisms in aquatic systems and became dangerous to all kinds of organisms, including

bivalves, fishes, and aquatic plants and finally is transferred to human life [3]. Lead in the divalent cation form Pb^{2+} can be transported into the intracellular part through the Ca^{2+} transport systems thereby might altering the calcium homeostasis [38], affecting different cellular functions and enzymatic activities and causing DNA damage [39]. To our knowledge, our study is the first to assess the redox status in the mantle and adductor muscles of *M. stultorum* after Pb Cl_2 exposure. Furthermore, among the established mechanisms of lead toxicity is its ability to induce oxidative stress following an overproduction of ROS. An imbalance between antioxidants and pro-oxidants is a result of this toxicity and causing an alteration in redox status, lipids peroxidation and protein oxidations [39-40]. Malondialdehyde (MDA) constitutes the final products of lipid peroxidation indicating the degradation of lipids; it is therefore used as a good biomarker of lipid damage in aquatic organisms [4]. The oxidation of lipids during our experiment was confirmed by significant increases in MDA levels in the mantle and adductor muscles in all animals treated groups. The lipid membranes are the primary targets of oxidative damage [42]. So, the membrane integrity and fluidity in the bivalve's cells is altered by the pro-oxidant effects of $PbCl_2$ which was proved by these raise of MDA levels. As well, these high MDA levels are accompanied by high and significant decreases of lipid quantities in both tissues mainly for the clams exposed to D2 and D3 which also demonstrated a significant and negative correlation with the lipid contents in both tissues.. Moreover, the hypothesis that the inorganic cations as Pb^{2+} can stimulate the lipid peroxidation processes through the oxidation of polyunsatured fatty acids (PUFA) is generally suggested [37]. Indeed, these PUFA are extremely sensitive to oxidation via ROS's attack due to their high number of double bonds per fatty acid molecule [43]. In this context, the richness of mantle and adductor muscles by PUFA recorded by Chetoui *et al.* [9]can explain these low levels of lipids content and high MDA levels which are necessary the results of the toxic effects of lead [29,44].

Additionally, the generation of ROS is the main consequence of protein damage [45]. In our experiment, increases of AOPP levels are recorded in all *M. stultorun* treated groups for mantle and in *M. stultorun* treated with D2 and D3 for adductor muscle suggested that the harmful effects of Pb accumulation are leading to excessive protein oxidation. This oxidation of protein in the adductor muscles was associated with a remarkable and significant decrease in the amount of total protein during the treatment which probably due to their richness of total proteins compared to mantle [10]. These declines of protein quantities in AM are negatively correlated with the protein contents in this tissue. However, in the mantle, the total protein has not changed during the treatment. Previous research has shown that the alteration of protein in other tissues of *M. corallina* and *Venus verrcosa* are observed following the lead toxicity [16,46]. Similar decreases of protein content have been demonstrated in oyster gills and mussel digestive glands after metals exposure [43].

It is widely reported that the induction of MT's in marine organisms by metals is the result of their use as metal-pollution biomarkers [47]. These sulfhydryl groups (-SH) are involved in the detoxification processes [26]. And by capturing free radicals, these metalloproteins were able to acquire protective activity [48]. Our data showed that treatment with high Pb concentrations (5mg/L) leads to an increase of MT's content in the mantle tissue which is highly and positively correlated MDA and AOPP levels. However, we revealed that total Metallothionein (MTs) contents in adductor muscle enhanced in a dose-dependent manner which demonstrated a high and positive correlation with proteins and MDA levels. These MT's inductions reflected the impairment of both tissues functions in *M. stultorum*. Moreover, by comparing to the adductor muscles, this protein (MT's) is more active (or more expressed) in the mantle for each treated group. According to Kumari [49], the increase in MT concentrations seems to be a result of the increased transcriptions. And this suggestion has been demonstrated in *M. veneriformis* following mercury exposure which the basal MvMT mRNA expression was according to the ranking of the tissues like the following: digestive gland> mantle> gill>adductor muscle>foot [50]. Besides, these high MT's induction in the mantle than in the adductor muscles in our study can probably be firstly by the possibility of that tissue to accumulate more quantity of metals than the adductor muscle [29,51,52]and secondly by the activation of their main role in metal detoxification when the lipid peroxidation and protein oxidation reached the maximum. Our results are therefore in agreement with previous studies carried out on *Mytilus galloprovincialis* mantle and *M. corallina* tissues after lead exposure [15,16,29].

In the literature, the metals accumulated (as lead) in mantle and adductor muscles tissues seem to be lower than in the digestive gland and gills of *Venus veruscosa, Mytilus galloprovincialis, Callista chione, Perna viridis* and *Modiolus metcalfei* [29, 51,52]. Moreover, in our study, the stress biomarker response is clearly and significantly different between the two organs and appears to be more accentuated in the mantle of *M. stultorum* treated groups. These results can be explained that similar to gills, the mantle is also located in the mantle cavity which can directly interact with marine pollution and consequently can accumulate metals more than adductor muscle [29,51,52].

Conclusions

In conclusion, the present results demonstrate that $PbCl_2$ exposure (1mg / L; 2.5mg / L and 5mg / L) alters similarly the redox status of *M. stultorum* mantle and adductor muscles. The toxic effect of lead induces similarly the lipids peroxidation confirmed by the increases of MDA levels which they were associated with decreases in lipids contents in both tissues. The alteration of proteins expressed by the elevation of AOPP levels in both tissues confirms the harmful effects of lead. They were correlated with a decrease in protein content only in adductor muscles. The capacity of *M. stultorum* to increase their MT's

concentrations in both tissues seems to be an essential cellular adaptive system defending the animal against the lead-induced toxicity. These biomarkers responses in mantle and adductor muscle tissues elucidate the installation of oxidative stress by their increases in $PbCl_2$ treatment as compared to controls. However, they are distinctly different between mantle and adductor muscles. We can then deduce that the toxic effects of lead are greater in the mantle than in adductor muscles.

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